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(54) Title: PEPTIDES FOR DIAGNOSTICS AND THERAPEUTICS

(57) Abstract

This invention relates to peptides, polypeptides, or proteins or portions thereof, the amino acid sequences of which correspond to antigenic segments of an immunologically important protein of *Candida albicans*. These peptides, polypeptides or proteins are useful as diagnostic reagents for detecting the presence of antibodies reactive with *Candida Albicans* and may also be useful as therapeutic agents as well as immunogens in compositions and methods to illicit antibodies against *Candida albicans*.

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PEPTIDES FOR DIAGNOSTICS AND THERAPEUTICS

FIELD OF THE INVENTION

THIS INVENTION relates to peptides, polypeptides, or proteins or portions thereof, the amino acid sequences of which correspond to antigenic segments of an immunologically important protein of Candida albicans. These peptides, polypeptides or proteins are useful as diagnostic reagents for detecting the presence of antibodies reactive with Candida albicans and may also be useful as therapeutic agents as well as immunogens in compositions and methods to illicit antibodies against Candida albicans.

BACKGROUND OF THE INVENTION

Yeasts of the genus Candida are commensals or saprophytes of warm blooded animals. The Candida species are part of the normal endogenous microbial flora and are frequently present on the normal mucous membranes of the gastro- intestinal tract, upper respiratory tract, and genital tracts of humans. The yeast Candida albicans is an opportunistic pathogen which can exploit any weakness or disability in the body's immune defences to cause disease. It has few equals in terms of the variety and severity of infections for which it is responsible.

The most common infection caused by Candida albicans is thrush, a benign rash of the skin and mucous areas especially the throat and vagina. In addition, C. albicans infections are becoming increasingly common in association with HIV (Human Immunodeficiency Virus) infections.

The most severe form of C. albicans infection is systemic candidiasis. Systemic candidiasis has a mortality rate of over 70% amongst immunocompromised and debilitated patients (Gold, 1984). In these cases C. albicans overwhelms the already weakened body's defences and invades the tissues and organs. A severe infection results, which is frequently fatal.

Over the last few decades Candida species have assumed increasing prominence as major pathogens. The increase in Candida infections can be largely attributed to advances in modern medicine. The improved survival of patients with underlying neoplasms, collagen vascular

diseases, extensive trauma and other immunocompromising illness has increased the reservoir of patients at risk of Candida infections.

The number of patients undergoing organ and tissue transplants or chemotherapy for cancer has also increased, thus further adding to the reservoir of patients at risk of Candida infections. The immunosuppressive and antineoplastic drugs used in these patients, dramatically increases the risk of systemic candidiasis. It is estimated that 10% - 25% of leukaemia and transplant patients will develop systemic candidiasis.

Patients with intravenous catheters and other surgical devices which provide portals of entry of Candida into the body are also susceptible to systemic candidiasis. Candida species, predominantly C. albicans, are responsible for approximately 10% of all nosocomial infections (Edwards, 1991).

The use of oral contraceptives, broad spectrum antibiotics, hormonal therapies, drug abuse and the AIDS epidemic has brought about an increase in the incidence of mucocutaneous candidiasis. The significance of the frequency of mucocutaneous candidiasis in the general population is more difficult to determine as many cases are undetected or are not reported.

It is estimated that 75% of women will experience at least one clinically significant episode of vaginal candidiasis during their lifetime (Sobel, 1993). Based on the number of prescriptions written to treat C. albicans infections in the US from 1980-1990, the incidence of vaginal candidiasis has almost doubled (Kent, 1991). In 1990 over 13 000 000 US females received treatment for vaginal candidiasis.

The AIDS epidemic has been associated with a dramatic increase in the incidence of oesophageal and oral candidiasis, most HIV positive patients will suffer from oesophageal and oral candidiasis when they develop "full blown" AIDS.

Candidiasis is a world wide problem, with the most dramatic increase in incidence in "Westernised" countries. It is difficult to accurately estimate the number of individuals suffering from, or at risk from, candidiasis. However, based on the reported incidence of C.

albicans infections, the estimated number of infected individuals is likely to be several hundred million worldwide.

The diagnosis of mucocutaneous candidiasis is also hampered by the intrinsic nature of the infection. The relative lack of specificity of symptoms and signs precludes a diagnosis that is based upon clinical history and physical examination. C. albicans is frequently found as a harmless commensal of the vagina, respiratory and gastrointestinal tract and thus the culture of Candida from the oesophageal and vaginal secretions is not sufficient to establish a diagnosis of mucosal Candida infection. Further, the diagnosis of recurrent vaginal candidiasis is difficult since infections frequently occur in the absence of detectable levels of C. albicans.

Systemic candidiasis is difficult to diagnosis as patients show little in the way of objective clinical laboratory evidence of the nature or severity of the infection (Sugar, 1989). Often the only clinical indication of a systemic C. albicans infection is an elevated temperature which may be due to many other infectious agents in a severely ill patient. Culture techniques have been used for the laboratory diagnosis of C. albicans infections. However, positive cultures from urine, stool and respiratory specimens are difficulty to interpret as signs of deeper dissemination. The accuracy of systemic disease identification using positive cultures from multiple body sites has also been criticised (Sugar, 1989). Cultures of these types are negative in up to 56% of necropsy proven candidiasis cases (deRepentigny and Reiss, 1984).

The lack of a firm diagnosis for Candida infections makes clinicians reluctant to administer anti-Candida drugs which are often toxic to the patient and result in a number of side effects. Amphotericin is one such anti-Candida drug which is associated with pronounced nephrotoxicity (Seelig and Kozinn, 1982). In many cases the toxicity of anti- Candida drugs can contribute to the mortality of the patient. Also, by the time a firm clinical diagnosis of disseminated candidiasis has been obtained the infection has often progressed to such a stage that treatment is ineffective. The lack of early diagnosis and treatment has lead to a mortality rate from systemic candidiasis of over 70% (Meunier-Carpentier et al., 1981). There is, therefore much interest in developing a reliable sero-diagnostic test to allow for earlier detection and more effective treatment of systemic candidiasis.

Patients who are suffering from allergies, chronic fatigue syndrome, or who are generally unwell and thus run down are often falsely diagnosed as having C. albicans infections. A reliable sero-diagnostic assay will allow the more accurate diagnosis and effective treatment of Candida infections in these patients.

Current diagnostic and detection methods used for Candida infections are generally inadequate. The detection of Candida specific metabolites such as d-arabinitol in patients serum has been used to diagnose systemic candidiasis. Detection of d-arabinitol is achieved via the use of gas liquid chromatography or mass spectroscopy. The time, costs and complexity of these detection techniques makes the routine clinical application of d-arabinitol testing impractical. Further, in a recent study only 55% of 103 patients with systemic candidiasis had detectable serum levels of d-arabinitol before positive diagnosis by blood culture (Walsh et al., 1992).

One approach to the design of an effective diagnostic kit for the detection of Candida infections is the detection of circulating C. albicans antigens. These diagnostic methods suffer from a number of disadvantages including the fact that disseminated infections are often deep seated which results in a very low level of C. albicans antigens being present in the body fluids. Recent evaluations of diagnostic kits which rely on the detection of Candida antigens have cast doubt upon their value for diagnosis of systemic candidiasis (Phillips et al., 1990).

Other difficulties exist in the detection of circulating C. albicans antigens in the serum of infected patients. For example, early in the infection the amount of circulating antigen present in the serum of immunosuppressed patients may be insufficient to be detected by immunoassay. Later in the infection the presence of antibody antigen immune complexes in the serum may effectively inhibit the detection of the antigen by immunoassay.

Other diagnostic tests for infectious agents rely on the detection of specific antibodies synthesised by the host which are reactive to the infectious agent. These immunoassays have the advantage that they can be sensitive and specific and can be performed rapidly at a relatively low cost. Immunoassays such as immunoprecipitation, radio immunoassays (RAI) and enzyme-linked immunosorbent assays (ELISA) have been used for the detection of serum antibodies to mannan and cytoplasmic extracts of C. albicans (Ness et al., 1989). However, to

date all assays developed for the immuno-diagnosis of C. albicans antibodies have used complex antigen mixtures. As such the effectiveness of these tests is hampered by the fact that they contain components that can cross react with serum antibodies produced against other pathogens, hence current immunoassays detecting C. albicans antibodies have been criticised for their lack of sensitivity and specificity.

It has been identified that an alternative candidate for use in the diagnosis of fungal infections such as Candida is the detection of an antibody to a 48 kDa C. albicans antigen which has been shown in a significant proportion of patients suffering from systemic and mucocutaneous candidiasis. A drop in antibody titre to this antigen in the 48 kDa region has been associated with a poor prognosis for patients with systemic candidiasis. This antigen has been identified as the glycolytic enzyme enolase.

SUMMARY OF THE INVENTION

The present invention seeks to provide proteins, polypeptides, peptides or fragments corresponding to immunologically reactive portions of the protein enolase and processes for production thereof which may be useful in the diagnosis of Candida albicans infection and for use as a therapeutic agent and/or a vaccine in the treatment of the infection. It is envisaged that such proteins, polypeptides, peptides or fragments thereof may be used in diagnostic assays or as therapeutic agents and/or vaccines which have advantages over the diagnostic assays, vaccines and therapeutic agents presently available .

Thus, the invention provides a protein, polypeptide or peptide which is immunologically reactive with Candida albicans induced antibodies. Preferably, the protein, polypeptide or peptide is a recombinant protein, polypeptide or peptide. Further, it will be appreciated that the protein of the invention may be of various forms provided it is immunologically reactive with Candida albicans induced antibodies. In one preferred form, the protein is the protein enolase and more preferably the protein is the protein encoded by the amino acid sequence presented as Sequence ID No. 1.

The polypeptide of the invention may also be of various forms provided it is immunologically reactive with Candida albicans induced antibodies. In one preferred form the polypeptide is

provided by a portion of the protein enolase. Alternatively the polypeptide may comprise a portion of the amino acid sequence presented as Sequence ID No 1. In an especially preferred form the polypeptide comprises the polypeptide encoded by amino acid numbers 93 to 249 of the amino acid sequence presented as Sequence ID No 1.

The peptide of the invention may be of various forms provided it is immunologically reactive with Candida albicans antibodies. In one preferred form, the peptide comprises any one or more of the peptides encoded by any one or more of the amino acid sequences presented as Sequence ID No's 3 to 18.

In the sequences the single letter amino acid code is used wherein the letters mean:

A, Alanine; D, Aspartic acid; E, Glutamic acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arganine; S, Serine; T, Threonine: V, Valine; and Y, Tyrosine.

The present invention also provides a protein, polypeptide, or peptide of the general formulae X-Y-Z, wherein X and Z each represent independently of each other a hydrogen atom or a residue of an amino acid, of a protected amino acid, or a peptide, or of a polypeptide and Y represents one of the amino acid sequences presented as Sequence ID No 1 or a portion thereof or one of the amino acid sequences presented as Sequence ID No's 3 to 18 or portions thereof. Such sequences may be glycosylated or non glycosylated. In a preferred form at least one of X and Z comprises at least one of the sequences presented as Sequence ID No's 3 to 18 which may be the same as or different from Y.

The present invention also encompasses functionally equivalent proteins, polypeptides and peptides of the above proteins, polypeptides and peptides, which have at least a immunological property of the aforementioned sequences. For example proteins, polypeptides and peptides which have altered sequences which do not significantly affect the antigenic properties of the protein polypeptide or peptide are encompassed within the scope of the present invention. Further, it will be appreciated that when any of the terms protein, polypeptide or peptide are used, the terms are to be interpreted to encompass fragments,

functionally equivalent fragments, homologues and analogues of the protein, polypeptide or peptide.

The peptides encoded by the amino acid sequences presented as Sequence ID No. 1 and Sequence ID No's 3 to 18 are useful, alone or in combination, uncoupled or coupled to other molecules, in selective diagnostic methods for detecting fungal infections including those caused by Candida, as therapeutic agents for the treatment of Candida infections in immunisation against fungal infections including Candida infections, and in the production of polyclonal and monoclonal antibodies. Of course, their use is not to be limited to these methods.

It will be appreciated that the protein, polypeptide or peptide of the invention preferably comprises at least one epitope or antigenic determinant reactive with Candida albicans induced antibodies. The epitope or antigenic determinant is preferably reactive with enolase and even more preferably is reactive with Candida albicans enolase. The epitope may comprise at least one continuous (linear) epitope. Alternatively the epitope may comprise a conformational epitope.

It is also preferred that the protein, polypeptide or peptide provided by the invention comprise oligo peptides having the amino acid sequences presented as Sequence ID No. 3-18 containing therein sequences which comprise continuous (linear) epitopes reactive with Candida specific antibodies.

The present invention also provides DNA sequences and/or polynucleotide sequences coding for the proteins, polypeptides or peptides of the present invention.

In another form, the invention resides in an isolated and purified antibody, either monoclonal or polyclonal, induced by one or more of the proteins, polypeptides or peptides of the present invention.

The present invention also provides a process for the production of a protein, polypeptide or peptide comprising the steps of; culturing a host organism transformed with a vector

including a gene coding for a precursor of said protein, polypeptide or peptide; cleaving said precursor to produce said protein, polypeptide or peptide; and recovering said protein, polypeptide or peptide.

According to another form, the invention resides in a polynucleotide sequence, preferably a DNA sequence, coding for a protein polypeptide or peptide of the present invention. The polynucleotide preferably comprises the nucleotide sequence of Sequence ID No 2 or a portion thereof. DNA having this sequence may be obtained as described in the examples hereinafter and preferably the protein is Candida albicans enolase. The present invention also provides for an expression vector including the above polynucleotide or a portion thereof. The vector preferably includes the DNA sequence presented as Sequence ID No 2.

The DNA sequence presented as Sequence ID No 2 may also be used in the design of DNA probes for use in various hybridisation techniques to detect presence of fungal nucleic acid. Other segments of the DNA sequence presented as Sequence ID No. 2 may be useful as primers for use in Polymerase Chain Reaction (PCR) procedures enabling the specific amplification of fungal DNA sequences to aid their detection.

In yet another form, the invention resides in a therapeutic agent, a vaccine and/or pharmaceutical composition comprising an effective dosage of the protein, polypeptide or peptide of the present invention. for inducing protection in a living mammal against a fungal infection, or treating disease caused by a fungus, preferably the fungus is Candida albicans and preferably the fungal infection is caused by Candida albicans. In the case of the vaccine there may further be provided a pharmaceutically acceptable carrier.

The therapeutic agent, vaccine and/or pharmaceutical compositions may be formulated with a suitable pharmaceutical carrier and preferably contains a dosage of the protein enolase or a fragment or homologue or analogue thereof.

In yet another form the invention resides in a method for the in vivo production of antibodies against a protein, polypeptide or peptide comprising the step of contacting an antibody producing cell in vivo with said protein, polypeptide or peptide to raise said antibodies. In this form the protein, polypeptide or peptide may comprises at least one amino acid sequence

selected from an amino acid sequence presented as Sequence ID No's 3-18. Alternatively, the protein, polypeptide or peptide may comprise at least the sequence of Sequence ID No 1 or the protein enolase or a fragment or homologue or analogue thereof.

The present invention also provides a diagnostic kit for use in diagnosing Candida albicans infections comprising; at least one protein, polypeptide or peptide which is immunologically reactive with Candida albicans induced antibodies; means for detecting the binding of said antibodies to the protein, polypeptide or peptide. In one preferred form the protein, polypeptide or peptide is encoded by a portion of the amino acid sequence presented as Sequence ID No. 1. Preferably the portion comprises amino acids 93 to 249 of the amino acid sequence presented as Sequence ID No. 1. Alternatively, the protein, polypeptide or peptide utilised in the kit comprises the protein, polypeptide or peptide encoded by any one or more of the amino acid sequences presented as Sequence ID No's 3 to 18.

The present invention also provides a method of detecting Candida albicans induced antibodies in a sample comprising: reacting the sample with a protein , polypeptide or peptide of the invention and detecting the immunological complex.

DESCRIPTION OF THE INVENTION

One specific embodiment of the invention will now be described with reference to the drawings in which:

Fig 1 The identification of antigenic components of C. albicans by enzyme labelled anti-IgG antibodies by immunoblot analysis. Lanes 1-3 were incubated with human sera from vaginitis, normal and AIDS patients, respectively. Lane 4 was incubated with hyperimmune rabbit serum. Lanes 5 and 6 were incubated with affinity-purified antibody prepared against the 48 kDa antigen and the recombinant 48 kDa antigenic determinant, respectively.

Fig 2 An immunoblot of affinity-purified anti-48 kDa antigen antibody against SDS-PAGE fractionated proteins from lanes 1-4, C. albicans clinical isolates; lane 5, S. cerevisiae; lane 6, C. krusei; lane 7, C. tropicalis.

Fig 3 The location of the epitopes using a matrix plot of the individual peptide reactivity with four different sera samples from patients with vaginal candidiasis.

In order to detect the amino acid sequences corresponding to epitopes or antigenic determinants a differential screening strategy was used. A complete set of eight-mer amino acid sequences were synthesised such that subsequent sequences overlapped by six amino acids, and together the sequences covered the entire region of the enolase protein identified as the major IgG reactive region. Such extra amino acids are useful for coupling the sequences to each other, to another peptide, to a large carrier protein or to a solid support. Amino acids that are useful for these purposes include but are not limited to tyrosine, lysine, glutamine, aspartic acid, cysteine and derivatives thereof. Additional protein modification techniques may be used, example NH_2-Acetylation or COOH-terminal amidation, to provide additional means for coupling the peptides to another protein or peptide molecule or to a support.

As mentioned above also included are analogues or homologues of the amino acid sequence. Analogues are peptides which are functionally equivalent to the present peptides but which contain non naturally occurring amino acids. Homologues are peptides which have constitutively substituted amino acids which correspond to the peptides encoded by the genome of any other fungal isolates.

The polypeptides or peptides comprising an amino acid sequence (a) to (p) set out above can be used at least alone or in combination with other methods for detection of antibodies to Candida albicans. The antibodies thereby generated may also be used to detect Candida albicans associated antigens. Antibodies can be found in biological samples including but not limited to sera, other body fluids, tissue samples and other samples which may contain antibodies to Candida albicans. The peptides may also be useful as vaccines to protect against future infection by Candida albicans and/or as therapeutic agents to treat a Candida albicans infection.

The methods which use the peptides to detect the presence of Candida albicans specific antibodies in the sample involve contacting the sample with a peptide comprising at least one of the amino acid sequences (a) to (p) under conditions which allow the formation of an immunological complex between the peptide and any antibodies to Candida albicans that may be present in the sample. The formation of an immunological complex, if any, indicating the presence of antibodies to Candida albicans in the sample, is then detected and measured by suitable means.

Such detection methods include but are not limited to homogeneous and heterogeneous binding immuno assays, such as enzyme linked immunosorbant assays (ELISAs), radioimmuno assays (RIA), and Western Blot Analysis. Further assay protocols using the peptides allow for competitive and non competitive binding studies to be performed. The screening methods are rapid; efficient and allow for simultaneous screening of numerous samples.

The peptides may be labelled or unlabelled depending on the type of assay used. Labels which may be coupled to the peptides are those known in the art and include but are not limited to enzymes, radio nuclear nuclides, fluorogenic and chromogenic substrates, co-factors, biotin-avidin, colloidal gold and magnetic particles.

The peptides can be coupled by any means known in the art to other peptides, solid supports and carrier proteins. Such solid supports include but are not limited to polystyrene or polyvinyl microtitre plates, glass tubes or glass beads and chromatographic supports, such as paper, cellulose and cellulose derivatives, and silica. Carrier proteins include but are not limited to bovine serum albumen (BSA) and keyhole hemosianine (KLH).

Preferred assay techniques especially for large scale clinical screening of patient sera and blood and blood derived products are Agglutination, Western Blot techniques and ELISAs. ELISAs are particularly preferred for speed, the ability to test numerous samples simultaneously and ease of automation.

Samples including but not limited to body fluids and tissue samples, are then added to the peptides, where an immunological complex forms if antibodies to Candida albicans are

present in the sample. A signal generating means may be added to aid detection of complex formation. A detectable signal is produced if Candida albicans specific antibodies are present in the sample. Agglutination assays are commonly used and may utilise latex to which is bound to at least one of the peptides for use in a latex agglutination test. The coated latex beads can be mixed with a small volume of patient serum and examined for agglutination. If Candida albicans enolase specific antibodies are present in the patients serum agglutination (clumping) of the latex particles will be observed. While not as sensitive as an ELISA, latex agglutination assays are quick and easy to perform and would be suitable for medical practitioners or naturopaths as an initial screen.

An enzyme linked immunosorbent assay (ELISA) for the detection of antibodies to C. albicans is another use for the peptides of the current invention. The ELISA is a far more sensitive and quantifiable assay when compared with the latex agglutination assay. Although there are frequently low levels of antibody reactive with the C. albicans peptides in normal sera patients with an active C. albicans infection show a significant increase, relative to normal sera, in the titre of enolase specific antibodies. A standard titre of antibody reactive to the C. albicans enolase in normal healthy individuals would be determined, a titre of antibody above this level would be diagnostic of a C. albicans infection.

The ELISA test is based on techniques currently in use for detection of other antibodies. For use as reagents in these assays, the peptides of the invention are conveniently bonded to the inside surface of micro titre wells. The peptides may be directly bonded by hydrophobic interactions to the microtitre well, or attached covalently by means known in the art to a carrier protein, such as BSA, with the resulting conjugate being used to coat the wells.

As mentioned earlier the diagnosis of C. albicans infections in patients with systemic Candidiasis is difficult. These patients are often immunosuppressed and produce low levels of antibody which are unlikely to be detected in a latex agglutination test. In patients with systemic Candidiasis, a decrease in antibody titre is frequently associated with a poor prognosis. An ELISA enables the level of enolase specific antibodies to be monitored in these patients. The monitoring of the levels of C. albicans enolase specific antibodies over the period of an infection will facilitate a more rapid treatment of patients with C. albicans infections.

The peptides of the invention may also be formulated into pharmaceutical compositions or vaccines for use as immunogens. These immunogens can be used as or to elicit production of antibodies. The antibodies produced can be either polyclonal or monoclonal. For formulation of such compositions, an immunogenically effective amount of at least one of the peptides is admixed with a physiologically acceptable carrier suitable for administration to a subject. The peptides may be covalently attached to each other, to other peptides, to a protein carrier or to other carriers incorporated into liposomes or other such vessicles, or complexed with an adjuvant or absorbent as is known in the vaccine art.

Alternatively, the peptides are not complexed with the above and merely admixed with a physiologically acceptable carrier such as normal saline or a buffering compound suitable for administration to the subject. Methods of vaccine and antibody production, purification and characterisation are known in the art and will not be described in detail.

Antibodies made in response to the peptides and which recognise the peptides may also be formulated. Such antibodies can be either polyclonal or monoclonal. Methods for making antibodies are well known in the art.

The invention is further illustrated by the following specific examples which are not intended in any way to limit the scope of the invention in which:

Example 1 describes the preparation of a protein according to the invention.
Example 2 describes the determination of the complete nucleotide sequence according to the invention.
Example 3 describes the epitope mapping of a protein according to the invention, the preparation of particular peptide epitopes and their use in detecting Candida infections.

EXAMPLE 1

(a) Materials and Methods

(i) Yeast growth conditions

A clinical isolate of C. albicans (KEMH5) was obtained from the Department of Pathology, King Edward Memorial Hospital, Subiaco, Perth, WA. The yeast was grown overnight in YEPD (1% yeast extract, 2% peptone, 2% glucose) at 30°C with aeration).

(ii) Yeast antigen preparation

Candida albicans blastospores (10^9 cells) were resuspended in 1 mL of lysis buffer (PBS pH 7.4, 1 mmol/L phenyl methyl sulfonyl fluoride, 10 mmol/L β-mercaptoethanol, 1 mmol/L EDTA). An equal volume of 0.4 mm glass beads was added and the cells disrupted by vortexing at 4°C. The supernatant was decanted into a fresh tube and the glass beads were washed twice with 1mL of lysis buffer containing 0.1% SDS. The pooled supernatant was clarified by centrifugation (10 000g, 20 min) and the soluble protein content determined by SDS-PAGE electrophoresis.

(iii) Western blot analysis

A Candida albicans antigen preparation was solubilized and reduced by boiling for 5 min in 62 mmol/L Tris HCl, PH 6.8 containing 700 mmol/L β-mercaptoethanol, 10% (v/v) glycerol, 2% (w/v) SDS and 0.001% (w/v) bromophenol blue and then fractionated on a 7.5-12.5% PAGE gradient gel and electroblotted on to nitrocellulose membrane (Bio-Rad Laboratories Pty Ltd, USA). Protein molecular mass determinations were determined by comparison with molecular weight standards (Bio-Rad, USA) consisting of lysozyme, 14400 kDa; soybean trypsin inhibitor, 21500 kDa; carbonic anhydrase, 31000 kDa; ovalbumin, 45000 kDa; bovine serum albumin, 66200 kDa; and phosphorylase b, 92500 kDa.

The immunodetection was essentially performed as described by Mierendorf et al 1987, with the following modifications: free protein binding sites were blocked with

1% BSA in PBS- Tw (PBS pH 7.4, 0.05% Tween 20) at 25°C for 60 min. The nitrocellulose strips were washed three times for 5 min in PBS-Tw, then incubated with sera (primary antibody, 1/100 final dilution in PBS-Tw) at 25°C for 90 min. After a further three 5 min washes in PBS-Tw the strips were incubated with the relevant IgG-alkaline phosphatase conjugated secondary antibody (Silenus Laboratories Pty Ltd, Vic, Australia) at 25°C for 60 min. After three 5 min washes in PBS, the antigen-antibody complexes were detected by monitoring the deposition of purple stain following reaction with the substrates NBT and BCIP.

(iv) Human sera

Human sera containing antibodies to C. albicans was obtained from patients with a clinical history of vaginoses, some of whom had candida isolated from vaginal specimens. Sera from patients with other acute infectious diseases; from patients infected with the human immunodeficiency virus (HIV); and from patients with the acquired immune deficiency syndrome (AIDS), were obtained from Queen Elizabeth II Medical Centre, Perth, WA.

(v) Hyperimmune serum

Candida albicans proteins (200μg) were injected subcutaneously into a New Zealand white rabbit. A further injection was given 14 days later and serum was collected after 28 days. The serum contained high levels of Candida reactive/or specific antibodies as determined by Western blot analysis.

(vi) 48 kDa specific antibodies

The C. albicans antigen preparation was fractionated by SDS-PAGE and electroblotted on to nitrocellulose. The horizontal band containing the immunodominant antigen was excised, and affinity-purified antibody prepared as described by Matthews and Burnie, 1988.

(vii) Immunscreening of a C. albicans cDNA library

A C. albicans cDNA library constructed in the vector λgt11 was obtained from Clonetech Laboratories Inc. (Palo Alto, CA, USA). The λgt11 library was plated and the induced protein transferred to nitrocellulose discs. The immunoscreening protocol was the same as that used for electroblotted protein. The concentrations of the primary antibody (affinity purified antibody) and the secondary antibody were 1/500 and 1/3000, respectively.

(viii) Purification of recombinant protein

Protein was isolated from recombinant Escherichia coli clones as described by Huynh et al, 1985. A positive clone was selected and affinity-purified antibodies prepared as previously discussed.

(ix) DNA sequence analysis

λ DNA was purified from the positive clones and the cDNA insert from one clone excised and subcloned into M13mp18. The cDNA was sequenced in both orientations using the di- deoxychain termination method using a Taq polymerase DNA sequencing kit (Biotech International Limited, Perth, WA). The deduced amino acid sequence was used in a homology search against protein sequences in the PIR data base.

(b) Results

Immunoblot analysis of serum from patients with vaginal candidiasis demonstrated an antibody response to a protein of C. albicans with an apparent molecular mass of 48 kDa (Fig 1), as estimated from semi-logarithmic plots of the position of molecular weight markers on gradient gels. This antibody appeared to be identical to that found in sera from patients with HIV infection or AIDS (Fig 1). Antibody to the 48 kDa antigen was also identified in hyperimmune rabbit serum (Fig 1). The latter serum was used to prepare an antibody probe against the 48 kDa antigen, the specificity of which

was confirmed by immunoblotting against SDS-PAGE fractionated C. albicans protein (Fig 1). The affinity purified anti-48 kDa antibody also exhibited weak reactivity against a similar sized antigen present in C. krusei, C. tropicalis and Saccharomyces cerevisiae (Fig 2).

Immunoscreening of approximately 100 000 plaques from the cDNA library with the affinity purified anti-48 kDa antibody identified five positive clones. Nucleic acid hybridization showed that all five positive clones contained homologous cDNA inserts. One clone (λ1.1) was selected and monospecific sera prepared against the fusion protein by adsorbing hyperimmune rabbit sera to a strip of nitrocellulose containing the electroblotted fusion protein. The resultant monospecific sera, when used as an antibody probe against a Western blot of C. albicans protein, showed reactivity with the original 48 kDa antigen (Fig 1).

The nucleotide sequence of the 470 bp cDNA insert from the clone λ1.1 contained a single large translational open reading frame, in frame with the λgt11 encoded β-galactosidase gene. The 157 amino acid sequence encoded by the cDNA insert was used in a homology search with the Protein Information Resource (PIR) data base. A 74% amino acid identity was found with the corresponding region of enolase from the yeast S. cerevisiae and 56% identity with enolase from chicken skeletal muscle. The region of homology extends from amino acid position 93 to position 249 of the S. cerevisiae enolase (the carboxyl terminus being amino acid 433). The degree of amino acid identity between the enolase of C. albicans and that of S. cerevisiae is in agreement with the closer evolutionary relatedness of the two yeasts relative to chicken. When chemically conserved amino acid substitutions are taken into account, the S. cerevisiae and chicken skeletal muscle enolases exhibit homologies of 97 and 93% respectively.

EXAMPLE 2

(a) Materials and Methods

(i) Preparation and screening of the C. albicans genomic DNA library

C. albicans genomic DNA was extracted from a clinical isolate KEMH5 using the aqueous method of Cryer et al, 1975. A C. albicans genomic library was constructed in the vector λGEM12 (Promega), using a modification of the partial end fill method of Zabarovsky and Allikmets, 1986. C. albicans genomic DNA (2 μg) was partially digested with Sau3AI to generate 10-20 kb fragments. The Sau3AI-digested genomic DNA was partially end filled using E. coli DNA polymerase (Klenow fragment, 1 U/2mg DNA) with 50 uM dATP and dGTP for 30 min at room temperature. The λGEM12 DNA was digested with XhoI and the ends filled with dCTP and dTTP and ligated with the end-filled genomic DNA. Following packaging, the library was plated using standard methods. The C. albicans genomic DNA library was screened with the radiolabelled cDNA probe λ1.1, corresponding to codons 93-249 of the C. albicans enolase coding sequence. One clone, λgen 6.1, was characterised further.

(ii) DNA sequencing

The λgen 6.1 DNA was digested with various restriction enzymes. The restriction fragments were subcloned into M13mp18 and M13mp19 vectors. The recombinant plaques were hybridised with radiolabelled λ1.1 cDNA. Positive clones were selected and the DNA sequenced by the dideoxy-chain termination method. Clones containing overlapping restriction fragments were identified by hybridisation studies. Where necessary, oligonucleotide primers were synthesised and used as primers in a cycle-sequencing reaction (using a fmol™ sequencing kit, Promega) with λgen 6.1 DNA as the template.

(b) Results

(i) Isolation of the C. albicans enolase gene

A 470-bp cDNA was identified encoding the major IgG immunoreactive epitope(s). The deduced amino acid sequence of the cDNA clone had 80% identity with amino

acid positions 93-249 of enolase from the baker's yeast, S. cerevisiae. The cDNA was purified from the λgt11 DNA, radiolabelled, and used as a probe to screen a C. albicans genomic DNA library constructed in the vector λGEM12. Several positive plaques were identified. One clone, λgen 6.1 containing a 13-kb DNA insert, was chosen for further analysis.

(ii) Nucleotide sequence analysis of the C. albicans enolase gene

A number of overlapping restriction endonuclease fragments generated from the λgen 6.1 clone we subcloned into M13. The complete nucleotide sequence of the C. albicans enolase gene was determined from the overlapping M13 clones, in both orientations. The open reading frame encoded a polypeptide of 440 amino acids with a predicted molecular mass of 47.2 kDa.

EXAMPLE 3

(a) Materials and Methods

The 157 amino acid region of the enolase, as determined by the DNA sequence of the λgt11 clone λ1.1 was synthesised on polyethylene pins by the method of Geysen et al (1987). A complete set of 8-mer peptides were synthesised such that subsequent peptides overlapped by six amino acids.

Peptides coupled to the surface of the pins were tested against human sera for enolase immunoreactivity by an ELISA. The human serum samples were diluted 1/100 in 0.01 M PBS, pH 7.2 (2mM Na_2HPO_4,9mM NaH_2PO_4, 150mM NaCl) containing 2% (v/v) BSA, 0.1% (v/v) Tween 20 prior to use. Sheep anti-human alkaline phosphatase conjugated IgG antibody (Silenus) was diluted 1/1 500 in 0.01 M PBS, 2% (v/v) BSA, 0.1%(v/v) Tween 20 (pre-coat buffer) prior to use.

Immunodetection was performed by immersing the pins containing the immobilised peptides in the appropriate solutions in 96 well microtitre trays. The pins were

blocked in 200 µl of pre-coat buffer for 60 min at 25°C with gentle agitation. The pins were then incubated in 175 µl of diluted (1/100) human serum overnight at 4°C. The pins were washed four times in a bath of 0.01 M PBS for 10 min each at 25°C with gentle agitation. The pins were then incubated in 175 µl of diluted (1/1500) sheep anti- human alkaline phosphatase conjugated IgG antibody for 60 min at 25°C with gentle agitation.

Prior to immunodetection, the pins were re-washed four times in a bath of 0.01 M PBS for 10 min each at 25°C with gentle agitation. Detection of immunoreactive peptides was performed by incubating the pins in 150 µl of 1 mg/ml p- nitro-phenyl phosphate in 10 mM diethanolamine, pH 9.5 containing 0.5 mM $MgCl_2$. The plates were incubated for 10- 30 min at RT. The reaction was halted by removing the pins from the detection fluid. The plates were then read at 405 nm in a Titertek Multiskan plate reader. For each serum sample, a matrix plot of the individual peptide reactivity relative to each other peptides was determined.

Peptide bound antibody was removed by heat, denaturation and sonication. The pins were sonicated in 0.1 M PBS, containing 1% (v/v) SDS and 0.1% (v/v) β-mercaptoethanol for 10 min at 60°C. The pins were rinsed twice in distilled water at 60°C for 30 seconds, with a final wash in water at 60°C for at least 30 min with gentle agitation. The pins were then immersed in boiling methanol (60°C) for at least 15 seconds. The pin were then ready to be re-used for immunodetection studies.

(b) Results

Epitopes reactive to IgG contained within specific 8-mer peptides were identified by using a matrix plot of the individual peptide reactivity against human sera samples as depicted in Fig. 3.

It should be appreciated that various other changes and modifications may be made to the embodiments described without departing from the spirit and scope of the invention.

REFERENCES

1. DeRepentigny, L. and Reiss, E. 1984. Current trends in immunodiagnosis of candidiasis and aspergillosis. *Rev. Infect. Dis.* **6**: 301-312.

2. Edwards, J.E., (1991) *Invasive Candida* infections. *New Eng. J. Med.* **324**: 1060-1062

3. Geysen et al 1987.

4. Kent, H.L. 1991. Epidemioloagy of vaginitis. *Am. J. Obstet, Gynecol.* **165**: 1168-1176.

5. Meunier-Carpentier, F., Kiehn, T.E. and Armstrong, D. 1981 Fungaemia in the immunocomprised host. Changing patterns, antigenaemia, high mortality. *Am. J. Med.* 71:363-370.

6. Ness, M.J., Vaughan, W.P., and Woods, G.L. 1989. Candida Antigen Latex Text for Detection of Invasive Candidiasis in Immnocompromised Patients. *J. Infect. Dis.* **159**(3): 495-502.

7. Phillips, P., Dowd, A., Jewesson, P., Radigan, G., Tweeddale, M.G., Clarke, A., Geere, I., and Kelly, M. 1990. Nonvalue of antigen detection immunossays for diagnosis of candidemia. *J. Clin. Microbiol.* **28**(10): 2320-2326.

8. Seelig, M.S. and Kozinn, P.J. 1982 Clinical manifestations and management of candidosis in the compromised patient, p49-92. In D.W. Warnock and M.D. Richardson (ed). Fungal infection in the compromised patient. John Wiley & Sons, Inc., New York.

9. Sobel, J.D. 1993. Genital candidiasis. In: Candidiasis: Pathogenesis, diagnosis and treatment. Ed. G.P. Bodey. Raven Press Ltd. NY. pp225-247.

10. Sugar, A.M. 1989. Problems in the diagnosis of invasive candidiasis in the immunocompromised patient. In: Opportunistic fungal infections; Focus on Fluconazole. Ed. R.G. Richardson. International Congress and Symposium Series number 153.

11. Walsh, T.J., Lee, J.W., Schaufele, R. et al. (1992). Rapid detection of d-arabinatol for early diagnosis and therapeutic monitoring of invasive candidiasis. In: *Program and Abstracts of the Thirty-Second interscience conference on antimicrobial agents and Chemotherapy, Los Angeles, 1992. Abstract 1063. American Society for Microbiology,* Washington, DC.

SEQUENCE LISTING

General Information
APPLICANT
Name Curtin University of Technology

TITLE OF INVENTION: Peptides for Diagnostics and Therapeutics

NUMBER OF SEQUENCES: 18

SEQUENCE ID No 1

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G N P T V E V D F T T D K G L F 32

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E L R D G D K S K W L G K G V L 64

K A V A N V N D I I A P A L I K 80

A K I D V V D Q A K I D E F L L 96

S L D G T P N K S K L G A N A I 112

L G V S L A A A N A A A A A Q G 128

I P L Y K H I A N I S N A K K G 144

K F V L P V P F Q N V L N G G S 160

H A G G A L A F Q E F M I A P T 176

G V S T F S E A L R I G S E V Y 192

H N L K S L T K K K Y G Q S A G 208

N V G D E G G V A P D I K T P K 224

E A L D L I M D A I D K A G Y K 240

G K V G I A M D V A S S E F Y K 256

D G K Y D L D F K N P E S D P S 272

K W L S G P Q L A D L Y E Q L I 288

S E Y P I V S I E D P F A E D D 304

W D A W V H F F E R V G D K I Q 320

I V G D D L T V T N P T R I K T 336

A I E K K A A N A L L L K V N Q 352

I G T L T E S I Q A A N D S Y A 368

A G W G V M V S H R S G E T E D 384

T F I A D L S V G L R S G Q I K 400

T G A P A R S E R L A K L N Q I 416

L R I E E E L G S E A I Y A G K 432

D F Q K A S Q L 440

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SEQUENCE ID NO 4

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SEQUENCE ID NO 15

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SEQUENCE ID NO 16

H N L K

SEQUENCE ID NO 17

A P D I K T

SEQUENCE ID NO 18

K A G Y K

CLAIMS

1. An isolated and purified protein, polypeptide or peptide which is immunologically reactive with Candida albicans induced antibodies.

2. A protein, polypeptide or peptide of claim 1 comprising a recombinant protein, polypeptide or peptide.

3. A protein of claim 1 or claim 2 wherein the protein is enolase.

4. A protein of claim 3 wherein the enolase is encoded by the amino acid sequence presented as Sequence ID No. 1.

5. A polypeptide or peptide of claim 1 or claim 2 comprising a portion of the protein of any one of claim 3 or claim 4.

6. A polypeptide of claim 5 wherein the portion of the protein comprises the polypeptide encoded by amino acid numbers 93 to 249 of the amino acid sequence presented as Sequence ID No. 1.

7. A peptide of claim 5 wherein the portion of the protein comprises any one or more of the amino acid sequences presented as Sequence ID No. 3, Sequence ID No. 4, Sequence ID No. 5, Sequence ID No. 6, Sequence ID No. 7, Sequence ID No. 8, Sequence ID No. 9, Sequence ID No. 10, Sequence ID No. 11, Sequence ID No. 12, Sequence ID No. 13, Sequence ID No. 14, Sequence ID No.15, Sequence ID No. 16, Sequence ID No.17, and Sequence ID No.18.

8. A protein, polypeptide or peptide of claim 1 or claim 2 which is of the general formula X-Y-Z, wherein X and Z each represent independently of each other a hydrogen atom or a residue of an amino acid, of a protected amino acid, or a peptide, or of a polypeptide and Y represents any one or more of the amino acid sequences presented as Sequence ID No. 1, Sequence ID No. 3, Sequence ID No. 4, Sequence ID No. 5, Sequence ID

No. 6, Sequence ID No. 7, Sequence ID No. 8, Sequence ID No. 9, Sequence ID No. 10, Sequence ID No. 11, Sequence ID No. 12, Sequence ID No. 13, Sequence ID No. 14, Sequence ID No.15, Sequence ID No. 16, Sequence ID No.17, and Sequence ID No.18.

9. A protein, polypeptide or peptide of claim 8 wherein at least one of X and Z comprises at least one of the amino acid sequences presented as Sequence ID No. 1, Sequence ID No. 3, Sequence ID No. 4, Sequence ID No. 5, Sequence ID No. 6, Sequence ID No. 7, Sequence ID No. 8, Sequence ID No. 9, Sequence ID No. 10, Sequence ID No. 11, Sequence ID No. 12, Sequence ID No. 13, Sequence ID No. 14, Sequence ID No.15, Sequence ID No. 16, Sequence ID No.17, and Sequence ID No.18.

10. A protein, polypeptide or peptide of any one of claims 1 to 9 wherein the protein, polypeptide or peptide comprise at least one epitope reactive with Candida albicans induced antibodies.

11. A protein, polypeptide or peptide of claim 10 wherein the epitope is a continuous (linear) epitope.

12. A protein, polypeptide or peptide of claim 10 wherein the epitope is a conformational epitope.

13. An isolated and purified polynucleotide sequence coding for the protein, polypeptide or peptide of any one of claims 1 to 12.

14. A polynucleotide sequence of claim 13 wherein the polynucleotide sequence comprises a DNA sequence.

15. A polynucleotide sequence of claim 14 wherein the DNA sequence comprises the sequence presented as Sequence ID No. 2.

16. An expression vector including the polynucleotide of any one of claims 13 to 15.

17 An isolated and purified antibody to the protein, polypeptide or peptide of any one of claims 1 to 12.

18 An antibody of claim 17 wherein the antibody is a monoclonal antibody.

19. An antibody of claim 17 wherein the antibody is a polyclonal antibody.

20. A process for the production of a protein, polypeptide or peptide of any one of claims 1 to 12 which comprises: culturing a host organism including a gene coding for a precursor of said protein, polypeptide or peptide; cleaving said precursor to produce said protein, polypeptide or peptide and; recovering said protein, polypeptide or peptide.

21. A diagnostic kit for use in diagnosing Candida albicans infections comprising: at least one protein, polypeptide or peptide which is immunologically reactive with Candida albicans induced antibodies and; means for detecting the binding of said antibodies to the protein, polypeptide or peptide.

22. A kit of claim 21 wherein the protein is enolase.

23. A kit of claim 22 wherein the enolase is encoded by the amino acid sequence presented as Sequence ID No. 1.

24. A kit of claim 21 wherein the polypeptide or peptide comprises a portion of the protein of any one of claims 22 or 23.

25. A kit of claim 24, wherein the polypeptide comprises the polypeptide encoded by amino acid numbers 93 to 249 of the amino acid sequence presented as Sequence No. ID No.1.

26. A kit of claim 21 wherein the peptide comprises any one or more of the peptides encoded by the amino acid sequences presented as Sequence ID No. 3, Sequence ID No. 4,

Sequence ID No. 5, Sequence ID No. 6, Sequence ID No. 7, Sequence ID No. 8, Sequence ID No. 9, Sequence ID No. 10, Sequence ID No. 11, Sequence ID No. 12, Sequence ID No. 13, Sequence ID No. 14, Sequence ID No.15, Sequence ID No. 16, Sequence ID No.17, and Sequence ID No.18.

27. A method of detecting Candida albicans induced antibodies in a sample comprising: reacting the sample with any one or more of the proteins, polypeptides or peptides of any one of claims 1 to 12 and detecting the resulting immunological complex..

28. A therapeutic agent for the treatment of Candida albicans infections comprising any one or more of the proteins, polypeptides or peptides of any one of claims 1 to 12.

29. A vaccine for inducing protection in a human comprising any one or more of the proteins, polypeptides or peptides of any one of claims 1 to 12 and a pharmaceutically acceptable carrier.

30. A protein, polypeptide or peptide substantially as herein described with reference to any one of the examples.

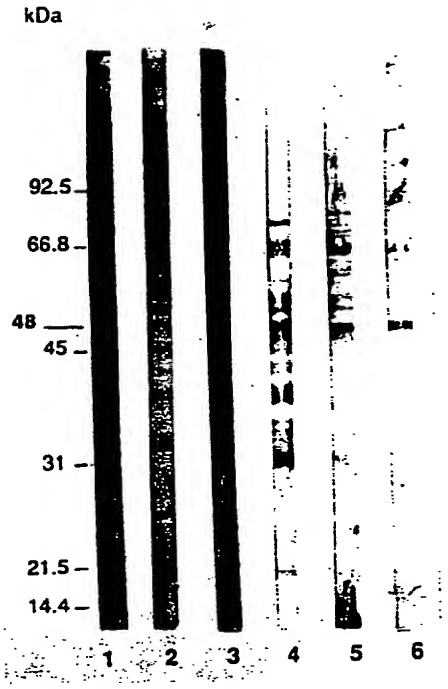


Figure 1

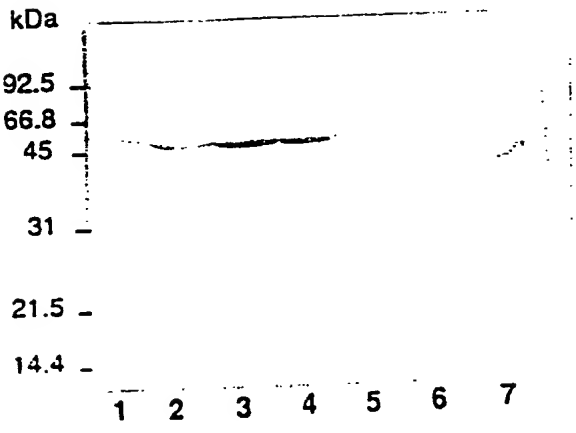
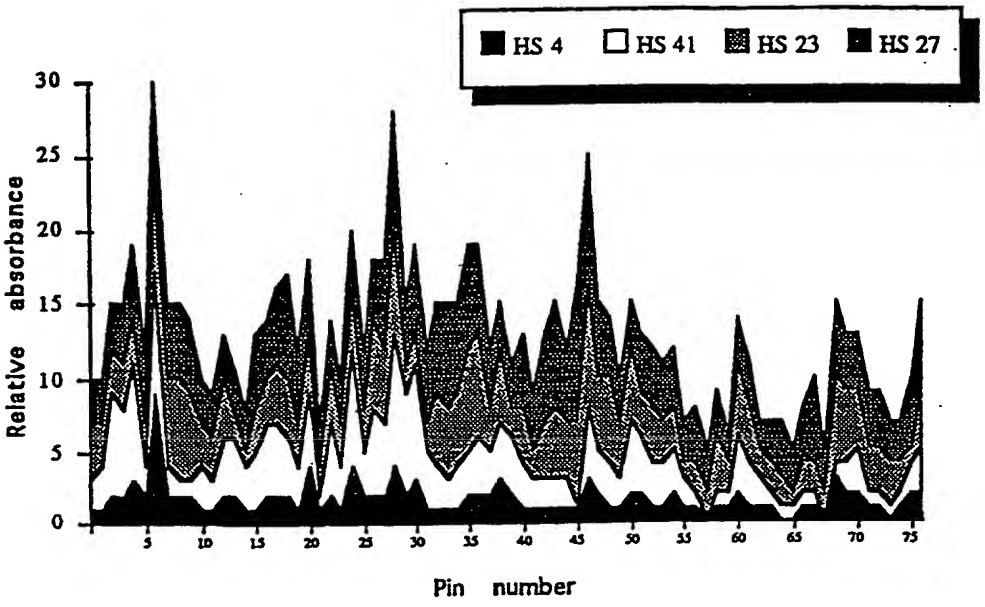


Figure 2



Pin Number	Peptide Sequence	Pin Number	Peptide Sequence	Pin Number	Peptide Sequence
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2	SLDGIPNK	28	PVPFQNVL	54	KKYGQSAG
3	DGIPNKSK	29	PFQNVLNG	55	YGQSAGKL
4	IPNKSKLG	30	QNVLNGGS	56	QSAGKLGD
5	NKSKLGAN	31	VLNGGSHA	57	AGKLGDEG
6	SKLGANAI	32	NGGSHAGG	58	KLGDEGGV
7	LGANAILG	33	GSHAGGAL	59	EDEGGVAP
8	ANAILGVS	34	HAGGALAF	60	EGGVAPDI
9	AILGVSLA	35	GGALAFQE	61	GVAPDIKT
10	LGVSLAAA	36	ALAFQEFM	62	APDIKTPK
11	VSLAAANA	37	AFQEFMIA	63	DIKTPKEA
12	LAAANAAA	38	QEFMIAPT	64	KTPKEALD
13	AANAAAAA	39	FMIAPTGV	65	PKEALDLI
14	NAAAAAQG	40	IAPTGVST	66	EALDLIMD
15	AAAAQGIP	41	PTGVSTFS	67	LDLIMDAI
16	AAQGIPLY	42	GVSTFSEA	68	LIMDAIDK
17	QGIPLYKH	43	STFSEALR	69	MDAIDKAG
18	IPLYKHIA	44	FSEALRIG	70	AIDKAGYK
19	LYKHIANI	45	EALRIGSE	71	DKAGYKGK
20	KHIANISN	46	LRIGSEVY	72	AGYKGKVG
21	IANISNAK	47	IGSEVYHN	73	YKGKVGIA
22	NISNAKKG	48	SEVYHNLK	74	GKVGIAMD
23	SNAKKGKF	49	VYHNLKSL	75	VGIAMDVA
24	AKKGKFVL	50	HNLKSLTK	76	GIAMDVAL
25	KGKFVLPV	51	LKSLTKKK		
26	KFVLPVPF	52	SLTKKKYG		

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 95/00176

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[6] C07K 14/40, 16/14, 16/40; C12N 15/13, 9/88, 15/60; G01N 33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
ELECTRONIC DATABASES: WPAT, JAPIO, KEYWORDS AS BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
ELECTRONIC DATABASES; STN, BIOT, USPM. KEYWORDS AS BELOW

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
STN; WPAT, JAPIO, BIOT, USPM. KEYWORDS: CANDIDA() ALBICANS(S) (EPITOPE# or ANTIBOD: or ANTI() BOD: or IMMUNOGEN:): ENOLASE# or PHOSPHO(S) PYRUVATE(S) HYDRATASE# or PHOSPHO(S) GLYCERATE(S) (DE() HYDRATASE# or DEHYDRATASE# or HYDROLYASE# or HYDRO()LYASE#)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	K. M. FRANKLYN and J R WARMINGTON: "Cloning and nucleotide sequence analysis of Candida albicans enolase gene". FEMS Microbiol Lett (1993), 111(1) 101-7	1-15
X	P SUNDSTROM and G R ALIAGA "Molecular cloning of cDNA and analysis of protein secondary structure of Candida albicans enolase, an abundant, immunodominant glycolytic enzyme". J Bacteriol (1992), 174(21) 6789-99	1-15
X	A. B. MASON, H. R. BUCKLEY and J. A. GORMAN "Molecular cloning and characterisation of the Candida albicans enolase gene". J Bacteriol (1993), 175(9), 2632-9	1-15

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 1995	10 July 1995 (10.07.95)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929	Authorized officer JOHN ASHMAN Telephone No. (06) 2832364